RECENT ADVANCES IN BREAST PATHOLOGY: MOLECULAR CLASSIFICATION AND TARGETED THERAPY

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ABSTRACT
The transformation of breast cancer pathology from a purely morphological, diagnosis-based approach to an approach that incorporates a molecular framework, based on gene expression arrays, immunohistochemistry markers and NGS for the molecular classification of cancers, for more specific and individual treatment decision making is now moving beyond doubt. Using intrinsic subtyping based on PAM50 and other classifiers, the traditional luminal, HER2-enriched and basal-like classifications have been improved, and this classification has found its way into clinically actionable therapy pathways. HER2-low and HER2-ultralow disease has become a new biological entity made possible by the advent of ADC in addition to the development of the CDK4/6 inhibitor combinations in the hormone receptor-positive disease, and the newly discovered subgroups of BRCA-mutated and triple-negative disease with PARP inhibitors and immune checkpoint blockade. Digital pathology and AI based image analysis is a growing tool that complements genomic profiling tests such as Oncotype DX and MammaPrint, to further enhance prognosis and minimise overtreatment with chemotherapy. Resistance, tumor heterogeneity and lack of equal access to molecular testing are still significant challenges to getting universal care. Spatial transcriptomics combined with liquid biopsy monitoring and pathology aided by artificial intelligence is the next promising decade of breast cancer care.

Keywords: Breast pathology, Molecular classification, HER2-low, Targeted therapy, Digital pathology.
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1. Introduction

Breast cancer is no longer viewed as one disease, but rather a heterogeneous group of molecularly heterogeneous entities with varying biological processes, response to treatment, and prognosis. Over the past decades, the main predictive tool was histopathological grading, determined largely by the three-plus-one criterion of tubule formation, nuclear pleomorphism and mitotic count, and only less radically by hormone receptor status. A more molecular classification system that classifies tumors by hormone receptor status, by human epidermal receptor 2 (HER2) expression and by gene expression that indicates their proliferation has complemented and in many cases replaced this morphology-based model. Such a change has allowed the design of targeted treatment that targets biological drivers found in the tumor (El Haddad *et al*., 2024) without using the same chemotherapy approach for two different types of tumors with just similar appearance. With the advent of immunohistochemistry, next-generation sequencing and digital image analysis, it is now possible for pathologists to characterise a tumour with ever increasing precision, directly influencing the choice of treatment, such as endocrine therapy in luminal disease, antibody-drug conjugates in HER2-low disease and immunotherapies in triple-negative breast cancer. Pathology reports have thus emerged from a descriptive document to an actionable decision-support tool, incorporating quantitative data from biomarkers that oncologists use at every stage of treatment, the path to treatment approach and the follow up for possible recurrence.

2. Molecular Subtyping Foundations

2.1. Intrinsic Subtypes and Gene Expression Profiling

Breast tumors are classified as Luminal A, Luminal B, HER2-enriched, or basal-like using a sensitive classification system, the PAM50, and intrinsic subtyping tools, which use expression patterns of 50 genes involved in hormone signaling and proliferation. These molecular subtyping groups show significant correlation with clinical outcome and largely supplanted the use of simple histological grade for prognosis, with some crucial instances of lack of agreement with immunohistochemical surrogates (Kang and Kim, 2024). Having high hormone receptor expression and low proliferation indices indicates good prognosis and is treated with endocrine monotherapy, whilst Luminal B tumors have low hormone receptor expression and high proliferation rates, and are therefore treated with combinations of targeted agents and/or chemotherapy. Basal-like is equivalent in many respects but not fully with clinically defined triple-negative disease and has been shown to have an aggressive disease course with a distinct mutational profile with increased mutations in the DNA repair related genes of BRCA.

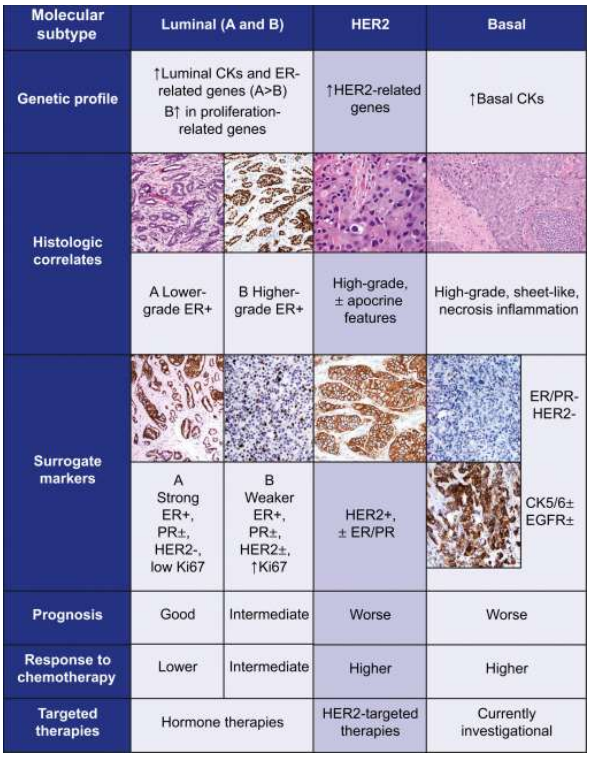

Figure 1: Molecular Classification of Breast Cancer

(Source: www.sciencedirect.com/topics/medicine-and-dentistry/breast-cancer-subtyping)

2.2. Immunohistochemical Surrogates

Immunohistochemistry (IHC) for estrogen receptor (ER), progesterone receptor (PR), and HER2 has been the workhorse of routine pathology and still serves as the key tool for assigning tumor subtypes due to the fact that genomic profiling is not universally available. With changes to the standardised scoring criteria, interobserver variability has been reduced, such as with HER2, where, unlike the binary positive-negative scoring which was formerly required, pathologists have to differentiate between HER2-zero, HER2-low, and HER2-ultralow status with greater reproducibility (Ivanova *et al*., 2024). Although the Ki-67 index is commonly used to approximate the plating efficiency of the cells, and to distinguish Luminal A from Luminal B disease, there remains a lot of potential for analytical variation between laboratories and many attempts are being made to standardise thresholds with the help of image analysis.

Subtype	**Receptor Profile**	**Typical Therapeutic Approach**
Luminal A	ER+/PR+, HER2-, low Ki-67	Endocrine therapy, CDK4/6 inhibitors
Luminal B	ER+/PR+/-, HER2+/-, high Ki-67	Endocrine therapy plus chemotherapy or targeted agents
HER2-enriched	HER2+, ER/PR variable	HER2-targeted antibody and ADC regimens
Basal-like/TNBC	ER-/PR-/HER2-	Chemotherapy, immunotherapy, PARP inhibitors

Table 1: Molecular Subtypes of Breast Cancer

(Source: Self-Created)

3. HER2 Reclassification and Antibody-Drug Conjugates

3.1. The HER2-Low and HER2-Ultralow Categories

One successful change in breast pathology in recent years has been the understanding that all HER2-negative tumors lie on a spectrum. HER2-low disease describes cases marked by immunohistochemistry (IHC) scores of 1+ or 2+ (phenotype 2) and whose in situ hybridisation (ISH) results were negative, representing about half of all breast cancers thought to be HER2 negative (phenotype 1) (Schmidt *et al*., 2024). New emerging data on HER2-ultralow expression, where there is less than 1+ staining in the membrane of the cell. This points to an even finer significance gradient, and therefore implications as to which patients may be best served with the newer antibody-drug conjugates. This reclassification has led to considerable training of the pathology lab, because

the criteria for scoring HER2 has originally been designed for binary (positive or negative) results, and are now expected to make an accurate diagnosis for more subtle cutoff levels, with direct consequences in downstream therapy.

3.2. Trastuzumab Deruxtecan and Related ADCs

A novel class of antibody-drug conjugates, that use a cytotoxic agent to directly attack tumour cells, takes advantage of low level HER2 expression on tumour cells that were previously deemed to be therapeutically insignificant. Preclinical and clinical trials have shown meaningful benefits in terms of progression-free survival (PFS) for trastuzumab deruxtecan treatment compared with standard chemotherapy (ST) in patients with HER2-low metastatic breast cancer, establishing a new therapeutic subdivision between the classical two HER2-positive and HER2-negative therapeutic paths. As the released cytolytic payload can freely influence the neighbouring tumour cells, even in cases of low and heterogeneous HER2 receptor positivity, the mechanism also involves a bystander effect, accounting for the efficacy even in low and heterogeneous receptor positive tumours (Roy *et al*., 2023). This step has added emphasis on making sure that the departments of pathology have accurate, repeatable low level HER2 results and that they are able to differentiate between a HER2-zero and HER2-low result because this can now mean that this entire class of drugs is the determining factor for eligibility.

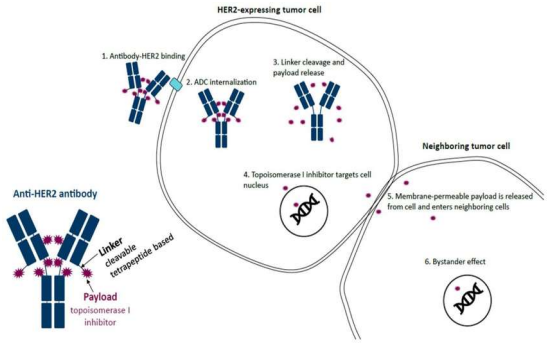

Figure 2: Mechanism of action of trastuzumab deruxtecan

(Source: www.researchgate.net/figure)

4. Hormone Receptor-Positive Disease and CDK4/6 Inhibition

4.1. Endocrine Therapy Backbone

Despite the advances in treatment for the past two or three decades, endocrine therapy is the foundation of systemic therapy for hormone receptor-positive, HER2-negative breast cancers. Depending on the stage of menopause, exposure to these drugs in the past, and potentially emerging mutations in the Aromatase seen on circulating tumor DNA assays, either aromatase inhibitors or selective estrogen receptor modulators or degraders (SERMs or SERD) are chosen. Aromatase inhibitors or selective estrogen receptor modulators or degraders (SERMs, SERD) are selected according to the menopausal status, previous treatment with these drugs and emerging resistance mutations identified by circulating tumor DNA assays (Shirman, 2023). Oral selective estrogen receptor degraders (SERD) are a newer therapy that is specifically active against ESR1 ligand-binding domain mutations which allow for resistance to standard aromatase inhibitors.

4.2. CDK4/6 Inhibitor Combinations

The emergence of CDK4/6 inhibitors with endocrine therapy is in the main line of treatment for advanced HRE+ disease and has been added in more and more high-risk early-stage situations. Such agents inhibit progression of the cell cycle at the G1-S arrest step, and a combination with hormone-blocking agents has not only greatly slowed down the progression of disease but also represents a significant challenge in clinical practice as acquired resistance through cyclin E overexpression, RB1 loss, and other pathway modifications still presents a diagnostic hurdle that must be addressed on an ongoing basis with the use of biomarkers (Morganti *et al*., 2024). Increased characterisation of proliferation markers and cell-cycle pathway status at relapse, requested by pathologists, is helping oncologists decide between continued CDK4/6 inhibition and a switch to an alternative mechanism in individual patients.

5. Triple-Negative Breast Cancer and Immune-Targeted Approaches

5.1. Molecular Heterogeneity Within TNBC

Triple-negative breast cancer is considered a distinct category because it does not express the receptors used to guide treatment for other breast cancer subtypes, but genomic profiling has identified three subgroups of triple-negative breast cancer: basal-like, mesenchymal and immunomodulatory. This heterogeneity has led to the search of other subclassifications of TNBC, where androgen receptor expression levels, BRCA mutation status and the density of tumor-infiltrating

lymphocytes (TILs) have been shown to predict optimal responses to specific therapies (Zhou *et al*., 2023). Recently, within TNBC, some proposed classifications break the tumor into some classes by the pathway activation that is dominant, such as the luminal androgen receptor subgroup, where activating AR signals are more prevalent and may require androgen receptor antagonist rather than traditional cytotoxic chemotherapy.

5.2. PARP Inhibitors and Immune Checkpoint Blockade

Patients with germline BRCA1 mutations and the BRCA2 mutations that are also enriched in triple-negative disease benefit from PARP inhibitors which exploit defects in homologous recombination DNA repair that are rendered sufficient with a synthetic lethality approach. Concurrently, PD-L1 expression has emerged as a companion diagnostic for immune checkpoint inhibitor eligibility, for example, in a metastatic triple-negative paradigm where biomarker testing guides, newly developed combination immunochemotherapy is now being used (Falato *et al*., 2023). Historically, tumour-infiltrating lymphocytes were used as a research tool; however, the number of lymphocytes isolated within a tumour is now beginning to be fed into pathology reports of triple-negative cancers, as higher density of lymphocytes has been shown to correlate with a better response to both chemotherapy and immune checkpoint blocking, giving a further biological dimension in addition to receptor status.

Marker	Associated Therapy Class
HER2-low/positive	Antibody-drug conjugates, monoclonal antibodies
BRCA1/2 mutation	PARP inhibitors
PD-L1 positivity	Immune checkpoint inhibitors
PIK3CA mutation	PI3K pathway inhibitors

Table 2: Targeted Agents by Molecular Marker in Breast Cancer

(Source: Self-Created)

6. Genomic Assays and Risk Stratification

6.1. Multigene Recurrence Score Panels

The Onco Tex assays can measure the proliferation and invasion-related genes that yield a recurrence risk score and are used to help determine whether chemotherapy should be combined with endocrine therapy in the early-stage hormone receptor-positive case. In patients that would benefit less from chemotherapy based on their clinicopathological data, these panels have significantly reduced unnecessary chemotherapy exposures (Wang *et al*., 2025), preserving chemotherapy toxicities from patients who would not otherwise have had these treatments based just on their clinicopathological data. These assays have been clinically validated in a number of large, prospective studies showing that low-risk-score patients can safely avoid chemotherapy without a compromise in the outcome of distant recurrence free survival.

6.2. Circulating Tumor DNA and Minimal Residual Disease

The incorporation of liquid biopsy into the routine follow-up of patients with breast cancer is gaining momentum, providing molecular surveillance of circulating tumor DNA (ctDNA) even before the radiological detection of recurrence or the detection of ESR1 alteration, type of resistance mutation that appears when the patient starts endocrine therapy, allowing to adapt the treatment earlier than imaging can (Uchikov *et al*., 2024). Patients who remain with non-detectable levels of ctDNA post-curative intent surgery are potentially eligible for treatment de-escalation, those with detectable levels might be eligible to consider escalation of therapy based on serial sampling to identify those at highest risk of relapse.

7. Digital Pathology and Artificial Intelligence

7.1. AI-Assisted Histopathological Analysis

The use of whole slide digital imaging and machine learning algorithms are making digitalisation affordable and feasible to automate and standardise Ki-67 quantification, HER2 scoring, and evaluation of tumor-infiltrating lymphocytes. This helps to minimise the inter-observer variability which has been one of the main factors affecting manual scoring reliability in histopathology of breast tissue (Nassar *et al*., 2026). These algorithms can analyse whole tissue sections at the cellular level, detecting subtle differences in levels of cellular staining that are more difficult to consistently assess by the naked eye, and therefore can directly assist in the evaluation of a new classification for treatment selection which are HER2-low and HER2-ultralow.

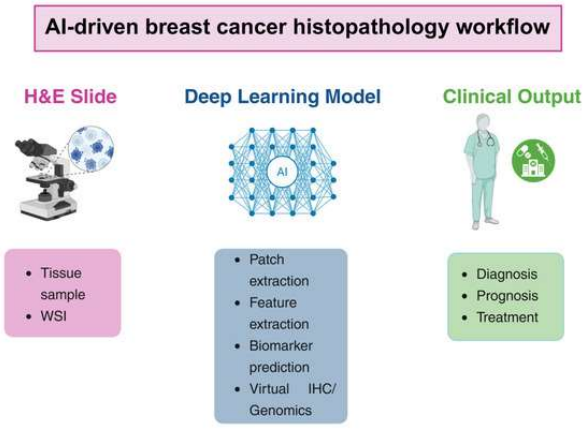

Figure 3: Integrating Artificial Intelligence into Breast Cancer Histopathology

(Source: www.mdpi.com/2072-6694/18/7/1184)

7.2. Integration with Multi-Omic Data

In line with the wider outlook of oncology, a trend of combining different types of data to develop an integrated risk model, digital pathology outputs are being combined with genomic and transcriptomic information. With this convergence, pathology labs can now deliver much deeper prognostic information from a single tissue sample than they can from any combination of individual modalities used separately, and preliminary research indicates the ability of image-derived features to predict genomic risk scores at half the cost of molecular standalone diagnostics, potentially opening a pathway for AI-sub-assisted pre-screening to direct resources toward those at highest risk of needing confirmatory genomic testing.

8. Challenges in Clinical Implementation

8.1. Tumor Heterogeneity and Testing Standardisation

The molecular classification process remains challenging due to intratumoral and intertumoral heterogeneity, and for HER2 and PD-L1, there is a range of scoring thresholds which may differ between laboratories, leading to inconsistent results in terms of assessing if they are HER2-low or HER2 PDL-1 low across institutions (McCaffrey *et al*. 2024). In the recurrent or metastatic setting, there is frequently discordance between the primary tumor microsatellite status and the microsatellite status of the metastatic site, further complicating treatment decisions, with a gradual shift in recommendations towards re-biopsying at the time of recurrence versus relying only on the primary tumor characterisation to inform recurrence treatment.

8.2. Access and Cost Barriers

Genomic testing and ADC remain costly and are not broadly available in all healthcare systems, leaving many, especially in lower resource areas, on empirical treatments instead of biomarker specific treatment. Newer restrictions on reimbursement of diagnostic testing (like the HER2-ultralow test) fall behind the landscape of the generation of clinical evidence, resulting in a disparity between the actual evidence-based practice recommendation and what is even practically available to patients outside the largest academic centers. This imbalance is exacerbated by differences in laboratory facilities, with careful low-level HER2 scoring, next generation sequencing and other advanced tests being performed in well-funded academic and urban institutions (Turner *et al*., 2023). However, in areas of low resource strength, it is common that community hospitals/clinics do not have the money or knowledge to set-up internal testing, requiring an appeal to send-out tests that contributes to delays in timely treatment decisions. This gap is further extended with insurance coverage, as newer companion diagnostic and ADC therapies are considered investigational long after approval for various length appeals processes and significant out-of-pocket expenses for patients (Fanucci and Mayer, 2024). These structural barriers must be overcome through coordinated efforts to reform the policy, to provide subsidised testing programs and to improve the reimbursement paths to make molecular advances a clinical benefit, not a privilege for a small group of patients.

9. Future Directions

Spatial transcriptomics is a trajectory of breast pathology that will lead to even more integration with spatial gene expression that can be understood as occurring within the architecture of the tissue, to go beyond extracting it from bulk sequencing alone, thereby mapping heterogeneity directly on histology. As targets for antibody-drug conjugates continue to develop beyond HER2 to include T-toxin, such as trophoblast cell-surface antigen, other emerging targets for payload delivery will make an increasingly larger population of patients become candidates for targeted, rather than cytotoxic, therapy (Amato *et al*., 2024). AI-driven pathology tools are not just ancillary research tools, but likely to be integral to standard practice in a workflow setting, while liquid biopsy based monitoring is likely to increase and become more widespread in real time during care across all molecular subtypes. The number of patients for whom a targeted therapy isn't available is expected to shrink even more, as synthetic and next-generation ADC payloads and additional immunotherapy combinations based on TIL signatures make their way into the clinic.

10. Conclusion

Breast pathology has passed from a simple morphology-based field to a receptor biology-based, genomic risk-based, and increasingly, digital and multi-omic approach. Refined understanding of the molecular basis of disease can directly impact treatment algorithms, such as reclassification of HER2 expression, drug maturation for the CDK4/6 inhibitors, and increasing eligibility for PARP inhibitors and immunotherapies in triple-negative disease (Schmid *et al*., 2024). Despite persistent challenges arising from the nature of tumour heterogeneity, the standardisation of testing, and equitable access to treatment, the overall direction of the advancement of breast cancer treatment and diagnostics is toward an individualised approach to cancer therapy, which would offer the pathology report as a dynamic, biologically informed decision support document, rather than a report that is a diagnostic label.

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